Supporting Information

Therapeutic Potential of Nitazoxanide; an Appropriate Choice for Repurposing versus SARS-CoV-2?

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Supporting Methods

FIGURES

Figure S1

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SARS CoV2 Antiviral Determination

Compound activity was assessed in 96-well plates using VERO E6 cells. Cells were seeded and allowed to reach 100% confluence overnight. Media was removed and cells treated with serially-diluted compounds in minimal medium at 25.00 µM, 8.33 µM, 2.78 µM, 0.93 µM, 0.31 µM, 0.10 µM and 0.03 µM or control media, as appropriate. DMSO was maintained at 0.25% for all experimental and control wells. The plates were then incubated at 37°C with 5% CO_2 for 2 hours. The minimal medium containing the experimental compounds and the control media was then removed. Wells were then treated with 50 µL minimal medium containing SARS- CoV-2 (MOI of 0.05; SARS-CoV-2/Human/Liverpool/REMRQ0001/2020), 100 µL 2× semi-solid media (EMEM supplemented with 4% HI FBS and 0.1% agarose) and then 50 µL minimal medium containing experimental compounds and control media added, as appropriate. After 48 hours, paraformaldehyde was added to each well (4% final concentration) and the plate incubated for 1 hour at room temperature. The medium was removed, cells were stained with crystal violet and washed three times with sterile water. Cytopathic viral activity was determined by measuring absorbance at 590 nm using a Varioskan LUX microplate reader (Thermo Fisher Scientific). Data were expressed as percentage inhibition of viral growth relative to the uninfected/untreated control (100% inhibition of viral growth) and the infected/untreated control (0% inhibition of viral growth).

Figure S1 Dose Response Curves for Nitazoxanise and Tizoxanide versus SARs CoV2 In vitro